



## **foodproof® GMO Bt176 Maize Quantification Kit**

### **Revision A, November 2023**

PCR kit for the quantitative detection of genetically modified maize event Bt176 using real-time PCR instruments.

### **Product No. KIT230044**

Kit for 128 reactions (lyophilized) for a maximum of 48 - 60 samples

Store the kit at -15 to -25 °C

For GMO testing purposes

**FOR *IN VITRO* USE ONLY**



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## 1. Product Overview

### 1.1 Number of Tests

The kit is designed for 128 reactions [i.e., 64 reactions with the GMO Gene Master Mix (vial 1, yellow cap) and 64 reactions with the Reference Gene Master Mix (vial 2, green cap)] with a final reaction volume of 25 µL each.

**Note:** The maximum number of samples that can be analyzed per experiment depends on the chosen quantification procedure:

Procedure A Including Relative Standard Curves	Procedure B Importing External Relative Standard Curves
Quantification of up to 48 samples (single sample preparation) in two runs plus calibration curves, one negative control reaction, and one calibrator in each run.	Quantification of up to 60 samples (single sample preparation) in two runs plus calibrator and one negative control reaction in each run.

### 1.2 Storage and Stability of Kit/Components

- Store the kit at -15 to -25 °C through the expiration date printed on the label.
- Once the kit is opened, store the kit components as described in the following kit contents table.

Vial	Label	Contents / Function / Storage
1 yellow cap	foodproof® GMO Bt176 Maize - GMO Gene Master Mix -	<ul style="list-style-type: none"> <li>• 2 x 420 µL</li> <li>• Ready-to-use primer and 5'-nuclease probe mix specific for the <i>cryIA(b)</i> gene of genetically modified Bt176 maize.</li> <li>• For amplification and detection of the <i>cryIA(b)</i> gene.</li> <li>• Store at -15 to -25 °C.</li> <li>• <b>Avoid repeated freezing and thawing!</b></li> <li>• <b>Protect from light!</b></li> </ul>
2 green cap	foodproof GMO Bt176 Maize - Reference Gene Master Mix -	<ul style="list-style-type: none"> <li>• 2 x 420 µL</li> <li>• Ready-to-use primer and 5'-nuclease probe mix specific for the starch synthase gene (<i>zSSIb</i>) of native maize</li> <li>• For amplification and detection of the <i>zSSIb</i> gene.</li> <li>• Store at -15 to -25 °C.</li> <li>• <b>Avoid repeated freezing and thawing!</b></li> <li>• <b>Protect from light!</b></li> </ul>
3 red cap	foodproof GMO Bt176 Maize - Enzyme Solution -	<ul style="list-style-type: none"> <li>• 4 x 32 µL</li> <li>• Contains Taq DNA Polymerase and Uracil-DNA N-Glycosylase (UNG, heat labile) for prevention of carry-over contamination.</li> <li>• Store at -15 to -25 °C.</li> </ul>
4 black cap	foodproof GMO Bt176 Maize - Dye Solution -	<ul style="list-style-type: none"> <li>• 4 x 32 µL</li> <li>• Contains a yellow dye for better visualization of the PCR mix in white PCR plates.</li> </ul>
5 purple cap	foodproof GMO Bt176 Maize - Calibrator DNA -	<ul style="list-style-type: none"> <li>• 2 x 50 µL</li> <li>• Contains a stabilized solution of plasmid DNA.</li> <li>• For use as a PCR run calibrator and positive control.</li> <li>• Store at -15 to -25 °C.</li> <li>• After first thawing, store at +2 °C to +8 °C for up to one month.</li> </ul>



Vial	Label	Contents / Function / Storage
6 blue cap	foodproof GMO Bt176 Maize - Dilution Buffer -	<ul style="list-style-type: none"> <li>• 4 x 1 mL</li> <li>• For dilution of calibrator and sample DNA.</li> <li>• Store at -15 to -25 °C.</li> </ul>
7 colorless cap	foodproof GMO Bt176 Maize - H <sub>2</sub> O PCR-grade -	<ul style="list-style-type: none"> <li>• 2 x 1 mL</li> <li>• Nuclease-free, PCR-grade H<sub>2</sub>O.</li> <li>• For use as a PCR run negative control.</li> <li>• Store at -15 to -25 °C.</li> </ul>

### 1.3 Product Description

The foodproof GMO Bt176 Maize Quantification Kit provides PCR primers and hydrolysis probes (5'-nuclease probes), and convenient premixed reagents for sequence-specific amplification and detection of the *cryIA(b)* gene (GM-specific DNA) and the maize *zSSI/b* gene (taxon-specific DNA). A Calibrator DNA is also provided to ensure accurate determination of the DNA copy number ratio of the GM-specific DNA to the taxon-specific DNA, expressed in %.

Results are obtained within 100 minutes. Optimized PCR conditions allow analysis of the GM-specific and the taxon-specific PCR in a single run. The Calibrator DNA provided with the kit serves as positive control and as a reference to normalize the relative DNA copy number ratio. Normalization corrects for differences in GMO content values, resulting from the combined variation in the quantity and quality of DNA samples and the efficiency of the PCR. The foodproof GMO Bt176 Maize Quantification Kit is specifically adapted for PCR using real-time PCR instruments.

- The GMO Gene Master Mix, (vial 1, yellow cap) allows the amplification and detection of a fragment of the synthetic *cryIA(b)* gene of genetically modified Bt176 maize using specific primers.
- A fragment of the native maize starch synthase gene (*zSSI/b*) is amplified and detected with the Reference Gene Master Mix (vial 2, green cap). The reaction product serves as a control for DNA integrity and as a reference for relative quantification.

**Note:** The kit described in this instruction manual has been developed for real-time PCR instruments.

### 1.4 Test Principle

Bt-176 maize is identified by detecting a DNA fragment of the synthetic *cryIA (b)* gene, which originates from the bacterium *Bacillus thuringiensis*. This gene is responsible for crop resistance to the European corn borer. To detect native maize, a maize-specific starch synthase gene (*zSSI/b*) is amplified and detected with the Reference Gene Detection Mix, which serves as both a control for DNA integrity and as a reference for relative quantification. Both amplicons are detected in separate reactions with specific pairs of primers and hydrolysis probes (5'-nuclease probes) using a real-time PCR instrument.

The basic steps of the test are as follows:

Step	Description
1	Using the kit's supplied sequence-specific primers in a polymerase chain reaction (PCR), the real-time PCR instrument and its associated reagents amplify and simultaneously detect fragments of genetically modified Bt176 maize and native maize, respectively.
2	The PCR instrument detects these amplified fragments in real time through fluorescence generated by cleavage of the hybridized probe due to the 5'-nuclease activity of the Taq DNA polymerase. The probe is labeled at the 5'-end with a reporter fluorophore and at the 3'-end with a quencher.



3	During the annealing/elongation phase of each PCR cycle, the probe hybridizes to an internal amplicon sequence downstream from one of the primer sites and is degraded by the 5'-nuclease activity of the Taq DNA polymerase. This cleavage of the probe separates the reporter dye from the quencher dye, increasing the reporter dye signal.
4	The real-time PCR instrument measures the emitted fluorescence of the reporter dye.
5	Determination of the relative DNA copy number ratio of the genetically modified Bt176 maize event. This can be performed using one of the following alternative procedures: <ul style="list-style-type: none"> <li>• Procedure A: Including relative standard curves into the run.</li> <li>• Procedure B: Importing external relative standard curve from a previous run.</li> </ul>

### 1.5 Application

The foodproof **GMO Bt176 Maize Quantification Kit** is intended for **GMO testing purposes only**.

The foodproof GMO Bt176 Maize Quantification Kit is used to determine the relative DNA copy number ratio of genetically modified Bt176 maize in flour or food. Relative quantification is performed by comparing the amplification of a specific gene of genetically modified Bt176 maize with the amplification of a specific gene of native maize. For calculation purposes, included or external (imported) standard curves can be used.

**Note:** The foodproof GMO Bt176 Maize Quantification Kit has been validated to quantify a DNA copy number ratio of up to 5 % GMO content.

### 1.6 Product Characteristics

<b>Specificity</b>	The primers and hydrolysis probes (5'-nuclease probes) provided in the GMO Gene Master Mix, (vial 1, yellow cap) and in the Reference Gene Master Mix, (vial 2, green cap) are sequence-specific for Bt176 maize and native maize, respectively.
<b>Sensitivity</b>	Detects the relative amount of 0.1% genetically modified Bt176 maize content in raw material.
<b>Measuring Range</b>	The kit can measure the relative content of Bt176 maize event in a range of 0-5 % (for standard material containing 0.1 % GMO, the coefficient of variation is equal to or less than 50 %; for standard material containing 1-5 % GMO, the coefficient of variation is equal to or less than 35 %).

### 1.7 Background Information

Real-time Polymerase Chain Reaction is commonly used to quantify GM fractions in food and feed samples. This DNA-based quantification technique measures the ratio between transgenic deoxyribonucleic acid (DNA), i.e., derived from the genetic modification, and endogenous DNA, which is specific for the biological species.

The genetically modified maize "Event 176" or "Maximizer", better known as Bt176 maize, was the first GMO maize approved in the EU [1]. Bt176 maize carries a gene that codes for the Cry1A(b) toxin, derived from the bacterium *Bacillus thuringiensis*, which confers resistance to the European corn borer. This plant pest leads to yield losses and high costs for crop protection. Additionally, Bt176 maize is herbicide-tolerant to glufosinate ammonium and carries a marker gene expressing resistance to the antibiotic ampicillin.

According to Swiss and EU laws [2-3], foods and additives with more than 1% and 0.9% relative amounts of genetically modified organisms (in Switzerland and European countries, respectively) must be labeled as GMO products.



**Note:** The value of the DNA ratio in reference materials may be not the same as the value of the certified powder mass fraction because of the different genetic composition of different parts of the seeds of monocotyledons (e.g., maize endosperm, seed coat and embryo). The mass fraction of a reference material expressed in g/kg or % takes the zygosity, ploidy and endoreduplication status of the seed used to produce the material not into account. In the European Regulation, the GM content is defined as the percentage of GM DNA copy number in relation to target taxon-specific DNA copy number, calculated in terms of haploid genomes [4]. The determination of the GMO content based on the number of copies of the target sequences per haploid genome will also be influenced by the zygosity of the maize event. This difference is normally expressed as the correction factor (CF).

## 2. Procedure

### 2.1 Before You Begin

#### 2.1.1 Precautions and Warnings

Quantification of the relative GMO content using the foodproof GMO Bt176 Maize Quantification Kit requires DNA amplification by PCR. The kit provides all required reagents in a ready-to-use master mix for the performance of PCR. However, in order to achieve reliable results, the entire assay procedure must be performed under nuclease-free conditions:

Prepare appropriate aliquots of the kit solutions and keep them separate from other reagents in the laboratory.

- Use nuclease-free labware (e.g., pipettes, pipette tips, reaction vials).
- Wear gloves when performing the assay.
- To avoid cross-contamination of samples and reagents, use fresh aerosol-preventive pipette tips.
- To avoid carry-over contamination, transfer the required solutions for one experiment into a fresh tube rather than directly pipetting from stock solutions.
- Physically separate the workplaces for DNA preparation, PCR setup and PCR to minimize the risk of carry-over contamination. Use a PCR-hood for all pipetting steps.
- In order to avoid cross-contamination, close all capillaries that contain sample DNA and negative controls before pipetting positive controls.

**Note:** Protect the GMO Gene Master Mix (vial 1, yellow cap) and the Reference Gene Master Mix (vial 2, green cap) from light.

#### 2.1.2 Additional Equipment and Reagents Required

- Real-time PCR instruments with a FAM detection channel.
- Real-time PCR compatible capillaries, tubes, strips or plates with optical cap or foil applicable for the PCR cyclers used.
- Standard benchtop microcentrifuge containing a rotor for 2.0 mL reaction tubes.
- Standard swing bucket centrifuge containing a rotor for multiwell plates with suitable adaptors.
- The LightCycler® Carousel-Based System provides adapters that allow LightCycler Capillaries to be centrifuged in a standard microcentrifuge rotor.
- *or*
- LC Carousel Centrifuge 2.0 for use with the LightCycler 2.0 Sample Carousel (optional).
  - If you use a LightCycler Instrument version below 2.0 you need in addition the LC Carousel Centrifuge 2.0 Bucket 2.1.
  - To adapt the LightCycler 2.0 Sample Carousel to the former LC Carousel Centrifuge, you need the LC Carousel Centrifuge 2.0 Rotor Set.
- Nuclease-free, aerosol-resistant pipette tips



- Pipettes
- Sterile reaction tubes for preparing PCR mixes and dilutions

**This kit must not be used in diagnostic procedures.**

The kit described in this Instruction Manual has been developed for real-time PCR instruments with a FAM detection channel. The performance of the kit was tested with the following real-time PCR instruments: LightCycler 480 II instrument, LightCycler 2.0 instrument and LightCycler 1.x instrument (Roche Diagnostics).

### 2.1.3 Sample Material

Use any sample material suitable for PCR in terms of purity, concentration, and absence of inhibitors. For preparation of genomic DNA from raw material of plant origin or from food, refer to the corresponding product package inserts of a suitable sample preparation kit (see Additional Equipment and Reagents Required).

**Note:** In order to quantify the relative amount of genetically modified maize in a sample of interest, the sample DNA must be diluted at least 1:4 in the Dilution Buffer (vial 6, blue cap) provided with the kit. This dilution step is essential to compensate for the different ion concentrations of the Calibrator DNA and the sample DNA. The latter depends on the buffers used for the sample preparation procedure. This dilution step also reduces the risk of inhibitory effects.

### 2.1.4 Assay Time

Procedure	Time
PCR Setup	15 min
PCR run	100 min (e.g., LC 480 II)
Total assay time	115 min

### 2.1.5 Positive Control

Always run a positive control with the samples. To prepare a positive control, replace the template DNA with the Calibrator DNA (vial 5, purple cap) or with a positive sample preparation control (e.g., Certified Reference Material).

### 2.1.6 Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with PCR-grade water (vial 7, colorless cap). Include a negative control during sample preparation to monitor reaction purity and cross-contamination. This extraction control can be used as an additional negative control reaction.



## 2.2 Program Setup

Program the PCR instrument before preparing the reaction mixes. The amplification is carried out according to the following temperature-time-program (for details on how to program the experimental protocol, see the operation manual of your real-time PCR cycler):

### Program:

<u>Pre-incubation</u>	<b>1 cycle</b>
Step 1:	37 °C for 4 minutes
Step 2:	95 °C for 10 minutes

<u>Amplification</u>	<b>50 cycles</b>
Step 1:	95 °C for 15 seconds
Step 2:	63 °C for 60 seconds

Fluorescence detection in step 2

For some real-time PCR instruments, the type of the probe quencher and the use of a passive reference dye has to be determined. The foodproof GMO Bt176 Maize Quantification Kit contains probes with TAMRA as a quencher and no passive reference dye.

**Note:** For users of the Agilent Mx3005P instrument:

Click 'Instrument → Filter Set Gain Settings' to open the Filter Set Gain Settings dialog box in which the gain settings may be viewed and modified. For FAM the Filter Set Gain Setting has to be modified to 'x1'.

## 2.3 Experimental Setup

### 2.3.1 General Remarks

Determination of the relative ratio content of Bt176 maize can be performed using one of the following alternative procedures:

- Procedure A: Quantification using included (in-run) relative standard curves
- Procedure B: Quantification using external (imported) relative standard curves

Thoroughly read the software instructions prior to performing this assay.

### 2.3.2 Procedure A - Quantification Using Included (In-Run) Relative Standard Curves

Each individual real-time PCR run consists of:

- Six dilution steps of the Calibrator DNA for both the GMO gene and the reference gene PCR in order to generate the respective calibrator curves (see table below),
- A variable number of sample preparations to be analyzed for genetically modified maize DNA amplification,
- At least one negative control reaction to control for contamination of the GMO gene and the Reference Gene PCR Master Mix, respectively.
- One positive reaction with the Calibrator DNA each for the GMO gene and the Reference PCR to compensate for constant differences between the PCR performance of the GMO gene and the reference gene.





Therefore, a typical experiment consists of 16 wells needed for controls, plus  $2 \times (n)$  wells needed for the samples of interest, where (n) indicates the number of food samples of interest. Since a multiwell plate has 96 wells, 40 food samples can be analyzed during one PCR run if the GMO gene and the reference gene are analyzed in the same run.

### 2.3.4 Dilution of Calibrator DNA

Quantification of the GMO content via procedure A requires the stepwise dilution of the Calibrator DNA (vial 5, purple cap) in the Dilution Buffer (vial 6, blue cap) as shown below:

Dilution step	Dilution	Concentrations to be Entered as Standards for the Reference Gene or the GMO Gene PCR
1	Undiluted	100
2	1:4	25
3	1:16	6.25
4	1:64	1.56
5	1:256	0.39
6	1:1024	0.098

### 2.3.5 Procedure B - Quantification Using External (Imported) Relative Standard Curves

Each individual real-time PCR run consists of:

- One positive reaction with the Calibrator DNA each for the GMO gene and the Reference gene PCR to compensate for constant differences between the PCR performance of the GMO gene and the reference gene,
- A variable number of sample preparations to be analyzed for genetically modified maize DNA amplification,
- At least one negative control reaction to control for contamination of the GMO gene and the Reference Gene PCR Master Mix, respectively.

Therefore, a typical experiment consists of 4 wells needed for controls plus  $2 \times (n)$  wells needed for the samples of interest, where (n) indicates the number of food samples of interest. Since a multiwell plate has 96 wells, 46 food samples can be analyzed during one PCR run if the GMO gene and the reference gene are analyzed in the same run.

**Note:** Procedure B is only applicable if the real-time PCR instrument used allows importing external standard curves generated in a previous run.



## 2.4 Preparation of the PCR Mixes

Proceed as described below to prepare a 20  $\mu\text{L}$  standard reaction. The PCR mixes for the GMO gene and the Reference gene must be set up separately, using the respective Master Mixes.

Do not touch the upper surface of the PCR plate.

1. Thaw the solutions and, for maximal recovery of contents, briefly spin vials in a microcentrifuge before opening. Mix carefully but thoroughly by pipetting up and down.
2. In a 1.5 mL reaction tube, prepare the PCR Mix by adding the following components in the order mentioned below, and then mix gently but thoroughly by pipetting up and down.

The volumes indicated below are based on a single 20  $\mu\text{L}$  standard reaction. Prepare the PCR mix by multiplying the amount in the "Volume" column by the number of reactions (sample and control reactions) to be cycled plus one or two additional reactions to cover pipetting losses.

Mix for the GMO Gene:

Component	Volume
GMO Gene Master Mix (vial 1, yellow cap)	13 $\mu\text{L}$
Enzyme Solution (vial 3, red cap)	1 $\mu\text{L}$
Dye Solution (vial 4, black cap)	1 $\mu\text{L}$
<b>Total volume</b>	<b>15 <math>\mu\text{L}</math></b>

Mix for the Reference Gene:

Component	Volume
Reference Gene Master Mix (vial 2, green cap)	13 $\mu\text{L}$
Enzyme Solution (vial 3, red cap)	1 $\mu\text{L}$
Dye Solution (vial 4, black cap)	1 $\mu\text{L}$
<b>Total volume</b>	<b>15 <math>\mu\text{L}</math></b>

3. Mix carefully but thoroughly by pipetting up and down. Do not vortex.
  - a. Pipet 15  $\mu\text{L}$  PCR mix into each well or capillary.
  - b. For the samples of interest, add up to 5  $\mu\text{L}$  sample DNA to a well or capillary (if less than 5  $\mu\text{L}$  add  $\text{H}_2\text{O}$  to 5  $\mu\text{L}$ ).
  - c. For the negative control, add 5  $\mu\text{L}$   $\text{H}_2\text{O}$ , PCR-grade (vial 7, colorless cap).
  - d. Procedure A: For the included (in-run) relative standard curves, add 5  $\mu\text{L}$  of each dilution of Calibrator DNA (vial 5, purple cap) to the wells or capillaries.
  - e. Procedure B: For the positive control, add 5  $\mu\text{L}$  Calibrator DNA (vial 5, purple cap) to a well or capillary.
4. Seal the plate or capillary accurately with an optical sealing foil or a stopper.
5. Place the plate in a swing bucket centrifuge and centrifuge at 1,500 x g for 30 s  
**Or** Place the adapters, containing the capillaries, into a standard benchtop microcentrifuge.  
**Note:** Place the centrifuge adapters in a balanced arrangement within the centrifuge. Centrifuge at 700 x g for 5 s (3000 rpm in a standard benchtop microcentrifuge).
6. Cycle the samples as described above.



## 2.5 Calculation of Relative Amount of Genetically Modified Maize

### 2.5.1 Procedure A – Quantification Using Included (In-Run) Relative Standard Curves

The use of calibration curves results in two values for every sample analyzed (i.e., one for the GMO gene and one for the reference gene).

**Note:** Since the calibration curves are specific for the GMO and reference PCR, respectively, it is important that the generated values for the GMO and reference PCR are distinguishable. The percentage of Bt176 maize relative to the total maize content within the sample of interest must be calculated manually, with a spreadsheet program or with the analysis software of the used real-time PCR instrument (e.g., LightCycler 480 instrument, Mx3005p system). Please refer to the manual for the real-time PCR instrument for more detailed information. The calculation of the relative GMO content is based upon the resulting crossing points or Ct-values of one particular sample and the efficiency of the PCR. In brief, the crossing point or Ct-value is the cycle at which PCR amplification begins its exponential phase and is considered the point that is most reliably proportional to the logarithm of the initial concentration. The efficiency of the PCR describes the kinetics during the reaction. The overall reaction efficiency is represented by the slope of the calibration curve. Since primers and hydrolysis probes (5'-nuclease probes) for both parameter-specific components (GMO and reference gene) have individual PCR efficiencies, a calibration curve for each gene must be generated.

#### Notes:

- Quantify two independent sample preparations of each food sample and take the mean value as the final result.
- The ratio of GMO:reference in the Calibrator DNA provided with the kit is 1.0.
- Due to statistical reasons, the Bt176 maize detection and quantification becomes less reliable at low copy numbers, (i.e., results obtained from sample material with crossing points or Ct values greater than 38 for either the GMO and/or the reference gene). Crossing points or Ct values greater than 29 in the reference PCR indicate there is not enough maize DNA in the sample to reliably quantify 1% GMO content.
- DNA degradation during food processing may affect GMO quantification.

### 2.5.2 Procedure B – Quantification Using External (Imported) Relative Standard Curves

For some real-time PCR instruments, it is possible to import external relative standard curves from a previously generated PCR run using the same instrument (e.g., LightCycler 480 instrument or LightCycler 2.0 instrument)

## 2.6 Related Procedures

### 2.6.1 Prevention of Carry-over Contamination

The heat-labile Uracil-DNA Glycosylase (UNG) is suitable for preventing carry-over contamination between PCR reactions. This technique relies on the incorporation of deoxyuridine triphosphate (dUTP) during all amplification reactions and the pretreatment of all successive PCR mixtures with the heat-labile UNG. The UNG cleaves DNA at any site where a deoxyuridine residue has been incorporated. The resulting abasic sites are hydrolyzed due to the high temperatures of the initial denaturation step and can no longer serve as PCR templates. The heat-labile UNG is inactivated during the initial denaturation step. Native DNA (e.g., the isolated genomic DNA from food or plant material) does not contain uracil and is therefore not degraded by this procedure. Since dTTP is replaced with dUTP and UNG is included in the foodproof GMO Bt176 Maize Quantification Kit, decontamination can be achieved with the provided reagents.



### 3. Appendix

#### 3.1 Troubleshooting

Observation	Possible Reason	Recommendation
No signal increase is observed, even with positive controls.	Incorrect detection channel has been chosen.	<ul style="list-style-type: none"> <li>Set Channel settings to FAM.</li> </ul>
	Pipetting errors or omitted reagents.	<ul style="list-style-type: none"> <li>Check for correct pipetting scheme and reaction setup. Repeat the PCR run.</li> <li>Always run a positive control along with your samples.</li> </ul>
	No data acquisition programmed.	<ul style="list-style-type: none"> <li>Check the cycle programs.</li> <li>Select acquisition mode "single" at the end of each annealing segment of the PCR program.</li> </ul>
	Inhibitory effects of the sample material (e.g., caused by insufficient purification).	<ul style="list-style-type: none"> <li>Use the recommended DNA sample preparation kit to purify template DNA.</li> <li>Dilute samples or pipet a lower amount of sample DNA (e.g., 2.5 <math>\mu</math>L instead of 5 <math>\mu</math>L).</li> </ul>
Fluorescence intensity is too low.	Inappropriate storage of kit components.	<ul style="list-style-type: none"> <li>Store the GMO Gene Master Mix, (vial 1, yellow cap) and the Reference Gene Master Mix, (vial 2, green cap) as indicated in Kit Contents Table, protect from light.</li> <li>Avoid repeated freezing and thawing.</li> </ul>
	GMO Gene Master Mix or Reference Gene Master Mix is not homogeneously mixed.	<ul style="list-style-type: none"> <li>Mix the GMO Gene Master Mix, (vial 1, yellow cap) and the Reference Gene Master Mix, (vial 2, green cap) thoroughly before pipetting.</li> </ul>
	Low initial amount of target DNA.	<ul style="list-style-type: none"> <li>Increase the amount of sample DNA. Depending on the chosen DNA isolation method, inhibitory effects may occur.</li> </ul>
Negative control samples are positive.	Carry-over contamination.	<ul style="list-style-type: none"> <li>Exchange all critical solutions.</li> <li>Repeat the complete experiment with fresh aliquots of all reagents.</li> <li>Always handle samples, kit components and consumables in accordance with commonly accepted practices to prevent carry-over contamination.</li> </ul>
Fluorescence intensity varies.	Insufficient centrifugation of the plate.	<ul style="list-style-type: none"> <li>Always centrifuge the plate as described.</li> </ul>
	Surface of the sealing foil is dirty (e.g., by direct skin contact).	<ul style="list-style-type: none"> <li>Always wear gloves when handling the plate.</li> </ul>



### 3.2 References

1. Commission Decision 97/98/EC of 23 January 1997 concerning the placing on the market of genetically modified maize (*Zea mays* L.) with the combined modification for insecticidal properties conferred by the Bt-endotoxin gene and increased tolerance to the herbicide glufosinate ammonium pursuant to Council Directive 90/220/EEC. Official Journal L 031, 01/02/1997 p. 0069–0070.
2. REGULATION (EC) No 1829/2003 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL OF 22 SEPTEMBER 2003, on genetically modified food and feed.
3. REGULATION (EC) No 1830/2003 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL OF 22 SEPTEMBER 2003, concerning the traceability and labeling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC.7.
4. European Commission Recommendation (EC) N° 787/2004 of 4.10.2004 on technical guidance for sampling and detection of genetically modified organisms and material produced from genetically modified organisms as or in products in the context of Regulation (EC) No 1830/2003. Off. J. Eur. Union L 348 (2004) 18-26.

## 4. Supplementary Information

### 4.1 Ordering Information

Hygiena Diagnostics GmbH is offering a broad range of reagents and services. For a complete overview and for more information, please visit our website at [www.hygiena.com](http://www.hygiena.com).

### 4.2 License Notice

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### 4.5 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: R 302 16



## 5. Change Index

### *Version 1*

First version of the package insert.

### *Version 2*

License Notice changed.

### *Revision A, November 2023:*

Rebranding and new layout.

R 302 16 20 -> INS-KIT230044-RevA



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